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(54) Device for disaggregating cytological specimens.

(57) A device for disaggregating cytological material contained within a sample vial and permitting the flow of the material to a tube. In order to obtain the disaggregation in shorter time and with less labour the device comprises:

a) an elongated hollow body (10) configured to be slidably inserted into the sample vial, the body (10) having a top end, a bottom end and sides;
b) sealing means (20) forming a liquid-impervious seal between the sample vial and the device when the device is inserted into the sample vial;
c) a plurality of shear plates (26, 36), at least one of the shear plates being disposed across the bottom end of the body (10), the shear plates (26, 36) defining a plurality of holes (28, 38) of pre-determined size effective to disaggregate the cytological material and to permit the disaggregated cytological material to traverse the shear plates (26, 36) and enter the body (10) when the body (10) is inserted into the sample vial; and
d) means (14, 16) for connecting the top end of the body (10) to the tube (17), permitting the disaggregated cytological material to flow from the body (10) into the tube (17).

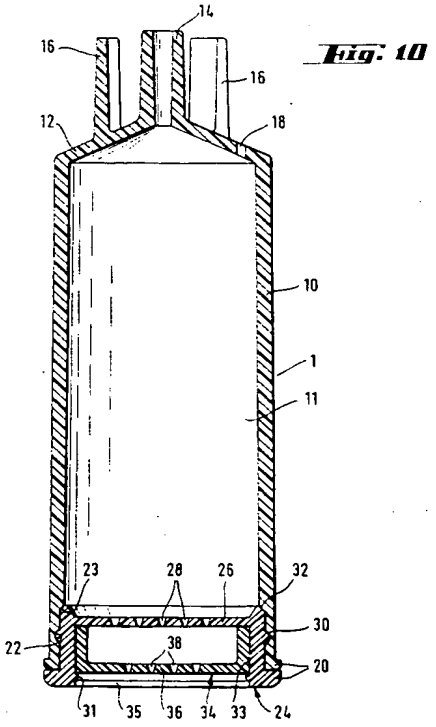

Fig. 10

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The invention relates to a device which disaggregates cytological material for specimen analysis.

Usually the cell suspensions received from doctors' clinics contain sample cells which have become aggregated or clumped together. To properly analyze this material, it is important to have a representative sample of single cells present on the microscope slide, with a minimum number of aggregated cell clumps. This is especially true for automated slide analysis.

A prerequisite for the screening of cytological material by an automated image analysis system is a reproducible and practical method for preparing smears of disaggregated cell suspensions. Many procedures have been described, for example, syringing, shaking, ultrasonic methods, and chemical methods, e.g., trypsinizing.

Of these, the most widely used is the syringing technique. This technique involves the use of a syringe typically having a 19-gauge needle. The cell suspension is drawn up into the syringe and then quickly expelled. The turbulent flow from the syringe shears and breaks up cell aggregates. This process is repeated many times, and can be done manually or automatically with a peristaltic pump. The difficulty with these techniques is that although effective, they are limited since they are labor-intensive and/or very time consuming.

In general terms, therefore, the aim of the invention is therefore to provide a device which makes it possible to prepare smears of disaggregated cell suspensions which are necessary for the screening of cytological material by an automated image analysis system. A particular aim of the invention is to provide a device of this kind which makes possible to prepare such smears in a shorter time and with less labor than with prior art devices.

According to the invention these aims are attained with a device which is characterized in that it comprises:

a) an elongated hollow body configured to be slidably inserted into the sample vial, the body having a top end, a bottom end and sides;

b) sealing means forming a liquid-impervious seal between the sample vial and the device when the device is inserted into the sample vial;

c) a plurality of shear plates, at least one of the shear plates being disposed across the bottom end of the body, the shear plates defining a plurality of holes of predetermined size effective to disaggregate the cytological material and to permit the disaggregated cytological material to traverse the shear plates and enter the body when the body is inserted into the sample vial; and

d) means for connecting the top end of the body to the tube, permitting the disaggregated cytological material to flow from the body into the tube.

In a preferred embodiment of the device according to the invention the bottom end portion of the body includes at least one shear plate preferably oriented substantially perpendicular to the longitudinal axis of the hollow body. The area located above the shear plate and confined within the sides of the hollow body defines a chamber for fluid containment. During use, the device is inserted into a specimen sample vial, so that the sealing means, such as an O-ring, fit snugly between the exterior surface of the device and the interior surface of the sample vial. This seal prevents the cell suspension from exiting into the area between the exterior wall of the device and the interior wall of the vial.

When the device is pushed into the sample vial, the cell suspension is forced through the shear plates and into the interior of the hollow body (chamber 11). For additional disaggregation, the device may be alternately withdrawn from and inserted into the vial to create a vacuum effect in the vial which volleys the cytological material between the chamber and the vial. This process may be repeated depending on the degree of disaggregation required. The disaggregated suspension within the chamber is then ready for subsequent use.

The device may also include a connector fitting to removably connect the top end of the device to a hose or other drainage tube. The device may be inverted together with the sample vial attached at the bottom end of the body. This inversion drains the disaggregated suspension from the chamber into a centrifuge tube or any other desired container via an opening in the top of the device. Preferably, a curved drainage tube is provided. The curved drainage tube contacts the inner surface of a centrifuge tube to permit a gentle flow of the disaggregated material, so as not to disturb a density gradient within the tube. To facilitate the inverted drainage, its useful to have a small hole in the body portion to permit an influx of air into the chamber without permitting an egress of liquid.

The invention is described hereinafter in terms of its preferred embodiments and with reference to the accompanying drawings in which:

Figure 1 is a top perspective view of a preferred embodiment of the invention.

Figure 2 is a side view of a preferred embodiment of the invention.

Figure 3 is a cross-sectional view of the device of Figure 1.

Figure 4 is a top view of a first shear plate.

Figure 5 is a side view of the assembly for the first shear plate shown in Figure 4.

Figure 6 shows a partial cross-sectional view of the first shear plate.

Figure 7 is a top view of a second shear plate.

Figure 8 is a side view of the assembly for the second shear plate shown in Figure 7.

Figure 9 shows a partial cross-sectional view of the second shear plate.

Figure 10 shows a cross-section of a preferred embodiment of the device 1 when completely assembled.

Figure 11 shows a side perspective view of a sample vial suitable for use with the subject invention.

Figure 12 illustrates a cross-sectional view of the device of Figure 10 partially inserted into a sample vial.

Figure 13 shows the same view as Figure 12 where the device is fully inserted into the vial.

Figure 14 illustrates draining of disaggregated suspension from a device according to the invention into a tube via a hose.

The invention is directed to a device for disaggregating cytological material. The term "disaggregating" refers to the separation of cells from large cell clusters into single cells or smaller clusters of cells.

Referring to Figure 2, device 1 is shown with body portion 10 which preferably is an elongated hollow member similar to the body of a syringe. More preferably, body 10 is predominantly configured as a hollow cylinder. Body 10 may be constructed as single unit, or may be made of multiple pieces which are assembled. The top end of body 10 may be open to the atmosphere, or preferably, may be formed with a tapered portion 12 as shown in Figure 2. Tapered portion 12 leads to the connector member 14 which may be used to removably connect the device 1 to a hose or other means for drainage of disaggregated cell suspension located in chamber 11. Preferably, the means for drainage includes a curved tube configured and dimensioned to contact the inside wall of a centrifuge tube to allow gentle layering of the cell suspension onto a density gradient.

Connector member 14 may be of any type which provides for quick removal, e.g. interlocking, or a simple male-female type connection, as shown. Also located on tapered portion 12 is securing means 16 which permits device 1 to be inverted and secured to a receptacle, such as a test tube or centrifuge tube (not shown) during drainage. Securing means 16 is designed to fit snugly against the interior wall of the receptacle. Alternatively, securing means 16 may be configured to fit snugly around the exterior wall of the receptacle to steady inverted device 1 in place during drainage. Securing means 16 shown in Figure 2 is a preferred embodiment of the invention and is designed to bias against the interior wall of the receptacle. Securing means 16, as shown, comprises three flanges equidistantly disposed about connector means 14. Securing means 16 could also take the form of a single flange which completely encircles the connector means 14. Any type of securing means which can securely hold the inverted device 1 to a receptacle opening is suitable for use in the invention.

Air vent 18 is provided through the wall of the device to permit the passage of air therethrough during either the filling of the device with cell suspension or during the drainage of the cell suspension from the device. The location of the air vent 18 is a matter of design choice.

Figure 2 also shows sealing member 20 located at the bottom end of the device. Sealing member 20 is typically an annular ring-shaped protrusion (similar in effect to an O-ring) which provides a substantially liquid-impervious seal around the exterior of the device when the device is inserted bottom end first into a sample vial. Sealing member 20 preferably is integrally formed with body 10 and/or with a shear plate member 24 described hereinafter. Alternatively, sealing member 20 may be a separate O-ring circumscribing body 10.

Figure 3 shows a cross-sectional view of the device of Figure 2. With the exception of annular groove 22, all elements illustrated in Figure 3 are also present in Figure 2 and have been previously described and explained. Annular groove 22, shown in Figure 3 proximate to the bottom end of body 10, receives annular ring 30 of a first shear plate member 24 (shown in Figures 4 to 6).

First shear plate member 24 is shown in Figures 4 to 6. In a preferred embodiment, first shear plate member 24 spans the entire cross-section of the body 10. Shear plate member 24 preferably includes annular ring 30 disposed around its exterior to connect with annular groove 22, providing an interlocking mechanism between first shear plate member 24 and cylindrical body 10. Any type of interlocking means may be used which removably connects first shear plate member 24 to cylindrical body 10. Indeed, the interlocking mechanism may take the form of threaded grooves, a mechanical interface, or the like. As shown in Figure 6, an additional annular ring 31 may be disposed or formed on inner wall 33 of shear plate member 24.

Annular ring 31 is designed to provide an interlocking mechanism with an additional or second shear plate member 34, which in a preferred embodiment, is inserted within opening 35. The positioning of first shear plate 26 is shown in Figure 6 at an upper location of the member 34, generally perpendicular to the longitudinal axis of cylindrical body 10. However, the invention also encompasses shear plate 26 placed at a location anywhere along the longitudinal axis of member 24. The second shear plate is shown in detail in Figures 7 to 9.

First shear plate member 24 includes shear plate 26 which comprises a plurality of holes or slots 28 formed therethrough for permitting passage of cytological material. The holes shown in Figure 4 are square in shape, however, holes of any shape may be used. Preferably, the holes are about 0.030 inch (760 micrometers) by 0.030 (760 micrometers) inch squares at their bottom edge. However, hole size in theory may vary from slightly larger than the cells to be disaggreated to a hundred times or more the cell size. Preferred diameter ranges for round holes are from about 300 micrometers to about 1,500 micrometers, more preferably from about 500 micrometers to about 1,000 micrometers, and most preferably about 800 micrometers.

To make the shear plates act like a perforated plane, the holes are typically tapered outward as they upwardly traverse the shear plate. The currently preferred outward taper is 15° relative to a plane drawn parallel to the longitudinal axis and tangential to the edge of the hole. Thus, the opening on the top of a shear plate is preferably larger than that on the bottom. The size and number and size of holes 28 and 38 may be optimized by one of ordinary skill in the art for the type of tissue being disaggregated and the level of disaggregation desired. First shear plate member 24 includes an angled edge 32 located at its upper most end. When shear plate member 24 is inserted into the bottom end of cylindrical body 10, the angled edge 32 abuts the edge 23 within the cylindrical body 10 ensuring a proper fit.

Figure 7 shows second shear plate member 34 including shear plate 36 which defines a plurality of holes or slots 38. In a similar manner to first shear plate 26, second shear plate 36 preferably covers the entire cross-section of the hollow body. Holes 38 can be of any size which permit passage of cytological material and promote the shearing action of the aggregated material into a generally single cellular material. However, holes 38 are preferably of the same size and shape as holes 28 of the first shear plate. Preferably, holes 28 and 38 on adjacent shear plates, e.g. 26 and 36, do not align. For the square matrix hole arrangement described below, a 45° rotation of one shear plate relative to the other appears to maximize the shear effect. It has been found advantageous to have a greater number of holes in the interior shear plates relative to the first exterior shear plate. Such a configuration prevents "spurting" of the cytological material to the top of body 10. Currently, it is preferred to have 24 holes 28 on first shear plate 26 and four holes 38 in second shear plate 36, first shear plate 26 having 24 holes 28 in a 5 x 5 square matrix, with the central hole in the matrix being absent, and second shear plate 36 having four holes 38 in a 2 x 2 square matrix. Although shear plates 26 and 36 may be spaced at any effective distance, it is currently preferred to space the plates at a distance of about 0.24 inches (0.61 cm) apart.

Shear plate member 34 is designed to fit snugly within the opening 35 of first shear plate member 24 with annular ring 31 holding second shear plate member 34 in place in opening 35. The positioning of second shear plate 36 is shown in Figure 7 at the bottommost location of member 34. However, the invention also encompasses shear plate 36 placed at a location anywhere along the longitudinal axis of member 34. The exact positioning of the first and second shear plates relative to each other is not critical. However, the plates should be generally parallel to each other and adjacent or proximate to each other such that a large percentage of the cytological material which passes through second shear plate 36 also passes through first shear plate 26 before entering chamber 11. The plates are typically placed as close as possible without impeding the flow of the cytological material therethrough.

It will be apparent to the ordinary artisan that any number of shear plates (limited by geometries and spatial considerations readily determinable by one skilled in the art) may be used in the device 1. The invention is not limited to the two shear plate members shown.

As an additional embodiment of the invention, the device may be provided with a mesh screen which spans the entire cross-section of the hollow body. The mesh is of a size to prevent particles of a size larger than a predetermined size from entering the chamber 11. If one shear plate is used in the invention, the mesh screen is preferably placed above the shear plate within chamber 11. If two or more shear plates are used, the mesh screen is preferably placed between the first and second shear plates. The mesh screen provides two important functions: (i) assisting in the disaggregation of the cell clumps and (ii) preventing material above a certain size to enter chamber 11. Preferably, mesh size is from about 80 to about 100 microns, but exact mesh size is a matter of design choice.

Figure 10 shows a cross-section of a preferred embodiment of the device 1 when completely assembled.

Figure 11 shows a side perspective view of the sample vial 41 as it would be received from a doctor's office or clinic. When received, sample vial 41 contains a sample containing fluid 45 and a volume of air 43 contained by cap 42. To begin the desegregation, a technician removes cap 42 from sample vial 41, so as to ready the sample for disaggregation.

Figure 12 shows a cross-sectional view of device 1 being inserted into sample vial 41. As

shown, device 1 is slidably inserted into sample vial 41 so that air 43, followed by sample containing fluid 45, is forced through second sheer plate 36, followed by first sheer plate 26, until the disaggregated material enters chamber 11.

Figure 13 illustrates device 1 completely inserted into sample vial 41. Sample fluid 45 (now disaggregated cytological material) is now within chamber 11. One preferred feature of the design is that device 1 does not contact the bottom of sample vial 41, because shoulder 44 of vial 41 (see Fig. 13) prevents device 1 from reaching the bottom of vial 41. This reserves an amount of fluid (typically about 2 mL) in the event the disaggregated material is wasted, thus allowing a second sample to be generated if need be. Withdrawal of device 1 from sample vial 41 causes a suction effect causing the sample fluid 45 to again traverse first sheer plate 26 and then second sheer plate 36 to reenter sample vial 41. By repeating this plunging activity, sample fluid 45 is volleyed between chamber 11 and sample vial 41 to further disaggregate the cytological material.

Once the disaggregated cytological material is contained within chamber 11, the combined device 1 and sample vial 41 maybe inverted as a single unit to allow the sample fluid 45 to drain via connector 14. As stated above, connector 14 is preferably coupled to a curved tube which is configured to allow gentle layering of the cell suspension onto a density gradient.

The above-described device can be manufactured from any type of material. In a preferred embodiment, the majority device is manufactured using low density polyethylene, and the shear plate is formed from polypropylene or other similar material. As apparent, the choice of material can be readily determined by a skilled artisan.

Having described the structure of the device, its use will now be described. Alter receiving a sample vial containing cytological material in physiological saline solution from a clinic, the laboratory technician inserts device 1 directly into the sample vial and exerts a downward force on the device to force cytological material upward through the shear plates. Downward movement of the device continues until the device has reached the bottom limit of movement in the vial. Alter a first passage, the technician may, at his or her option depending on the quality of disaggregation required, withdraw the device from the vial by pulling on it. The pulling action creates a vacuum-effect within the sample vial and draws the cytological material from the chamber 11 through the shear plates and back into the vial. The device is then ready to again be forced into the fluid to disaggregate the cells clumps. This procedure may be repeated as many times as is necessary to achieve the required degree of disaggregation.

Once the desired degree of cell clump disaggregation has been achieved, the cell suspension is then ready for subsequent processing. In a preferred embodiment, wherein a hose or tube is connected to the top end portion of the device as described above, and as shown in the Figures, the device may be removably secured through securing means 16, shown in Figure 2, to a receiving receptacle, e.g., test tube, centrifuge tube or the like (not shown), then inverted. The inverted device allows the cell suspension occupying chamber area 11 of cylindrical body 10 to drain directly into the receptacle for subsequent processing.

Figure 14 illustrates draining of disaggregated suspension from a device 1 according to the invention into a tube 17, e.g. a centrifuge tube, via a hose 19. Connector fitting formed by connector member 14 and flange 16 make it possible to removably connect the top end of device 1 to hose 19 or other similar drainage tube. The device 1 may be inverted together with the sample vial 41 attached at the bottom end of the body 10. This inversion drains the disaggregated suspension from the chamber 11 into a centrifuge tube 17 or any other desired container via an opening in the top of device 1. Preferably, a curved drainage hose 19 is used. The curved drainage hose contacts the inner surface of a centrifuge tube to permit a gentle flow of the disaggregated material, so as not to disturb a density gradient within the tube 17. To facilitate the inverted drainage, its useful to have a small hole 18 in the body portion of device 1 to permit an influx of air into chamber 11 without permitting an egress of liquid.

It will become apparent that other variations on the invention may be made without departing from the scope and spirit of the invention, defined in the appended claims.

Claims

1. A device for disaggregating cytological material contained within a sample vial and permitting the flow of the material to a tube, which comprises:
   a) an elongated hollow body (10) configured to be slidably inserted into the sample vial, the body (10) having a top end, a bottom end and sides;
   b) sealing means (20) forming a liquid-impervious seal between the sample vial and the device when the device is inserted into the sample vial;
   c) a plurality of shear plates (26, 36), at least one of the shear plates being disposed across the bottom end of the body (10), the shear plates (26,36) defining a plurality of

holes (28, 38) of predetermined size effective to disaggregate the cytological material and to permit the disaggregated cytological material to traverse the shear plates (26,36) and enter the body (10) when the body (10) is inserted into the sample vial; and
d) means (14,16) for connecting the top end of the body (10) to the tube (17), permitting the disaggregated cytological material to flow from the body (10) into the tube (17).

2. The device of claim 1, wherein the shear plates (26, 36) are positioned substantially perpendicular to the longitudinal axis of the body (10).

3. The device of claim 1, further comprising a mesh screen located adjacent to a shear plate, the mesh screen being configured to prevent cytological material larger than a size larger than a predetermined size from entering the body (10).

4. The device of claim 1, further comprising means (18) for selectively permitting gas to enter the body (10) while not permitting the liquid to exit the body (10).

5. The device of claim 1, wherein the securing means comprising a flange (16).

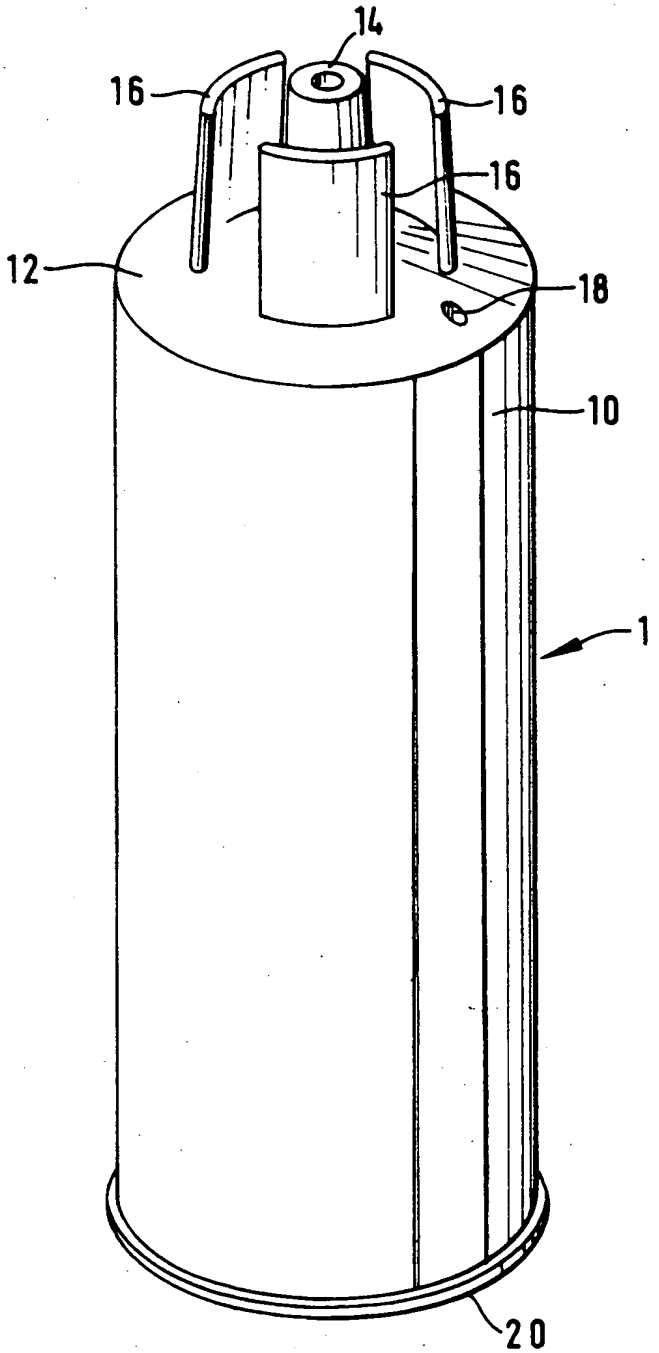
6. The device of claim 1, wherein the shear plates (26, 36) are configured so that the holes (28, 38) defined by adjacent shear plates (26, 36) are not in alignment.

7. The device of claim 1, wherein the sealing means is an annular protrusion (20).

8. The device of claim 2, comprising two shear plates (26, 36) arranged perpendicularly to the longitudinal axis of the body (10), the shear plates (26, 36) defining a plurality of holes (28,38) of predetermined size effective to disaggregate the cytological material and to permit the disaggregated cytological material to traverse the shear plates (26, 36) and to enter the body (10) when the body (10) is inserted into the sample vial, the positioning of the holes (28, 38) being such that they do not align and cannot be traversed by a line drawn parallel to the longitudinal axis of the body (10).

9. The device of claim 1, wherein the means for connecting the top end of the body (10) to the tube (17) comprise a curved hose (19) coupled to the top end of the body (10) so that the disaggregated cytological material is permitted to flow from the top end of the body (10) into the tube (17) via the curved hose (19).

Fig. 1



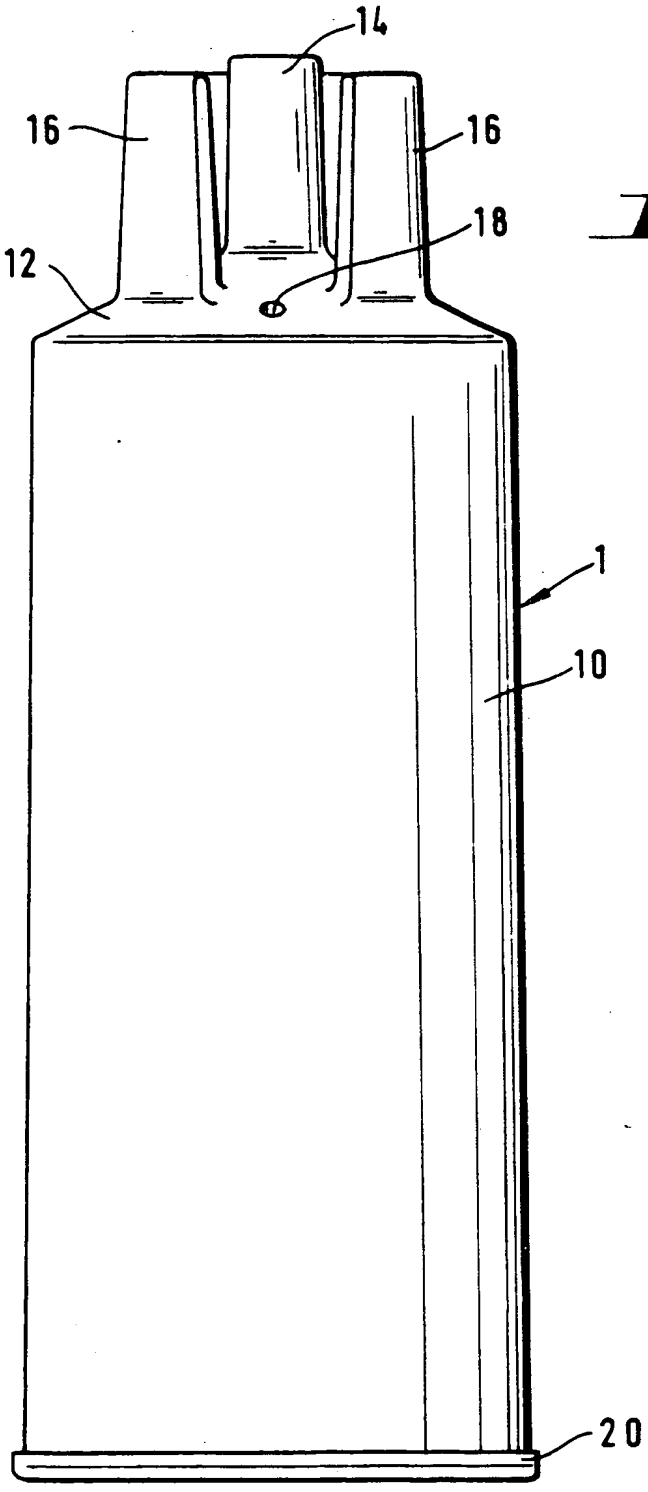


Fig. 2

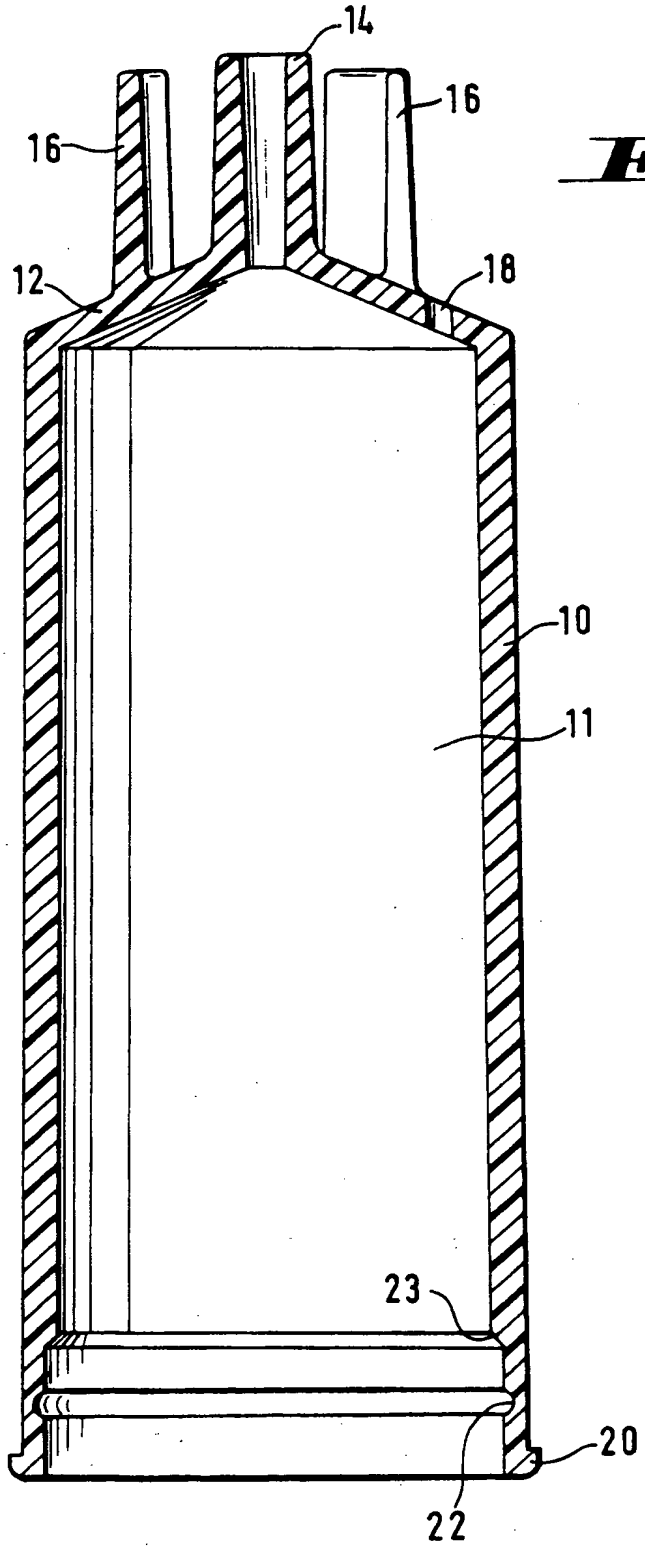


Fig. 3

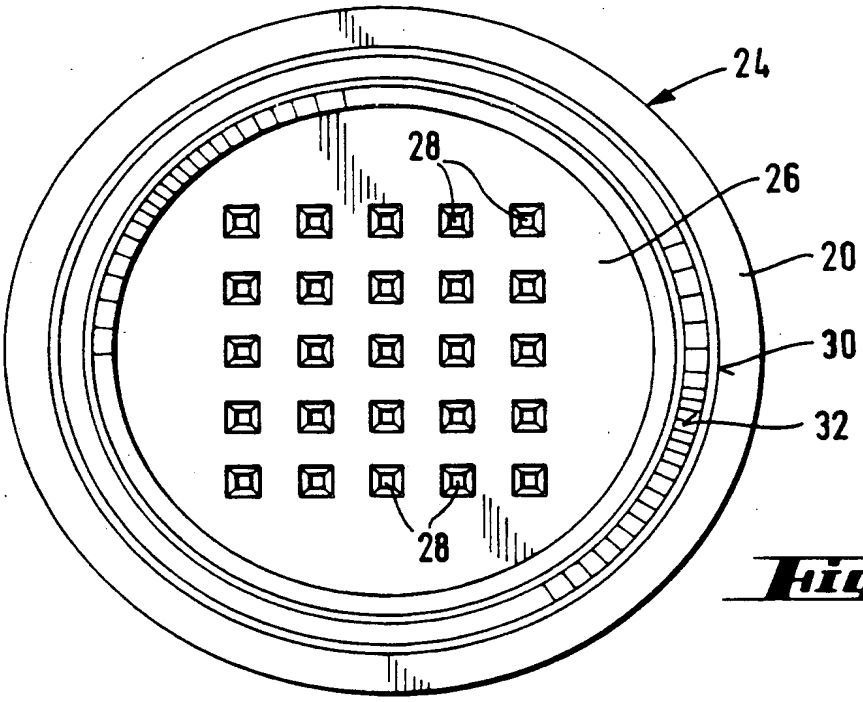


Fig. 4

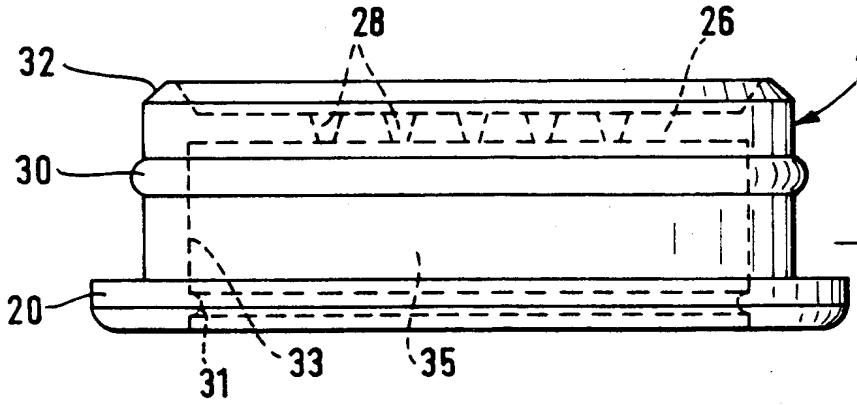


Fig. 5

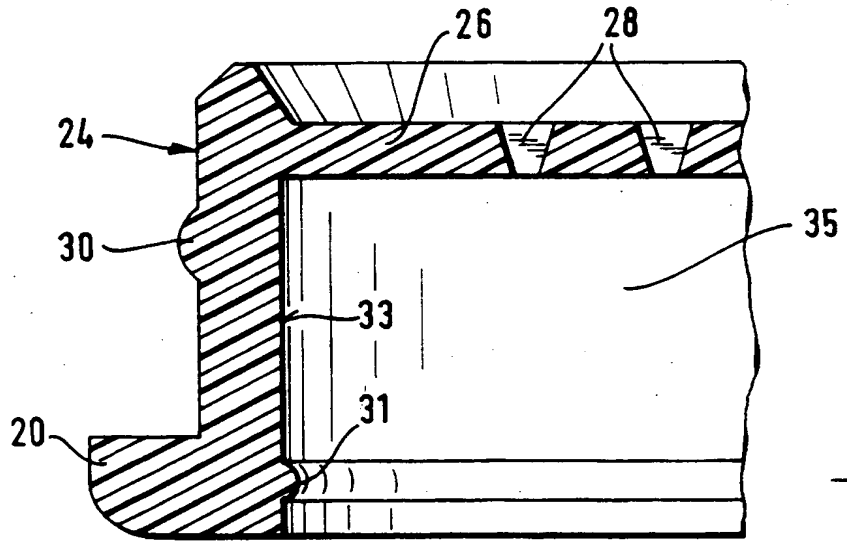
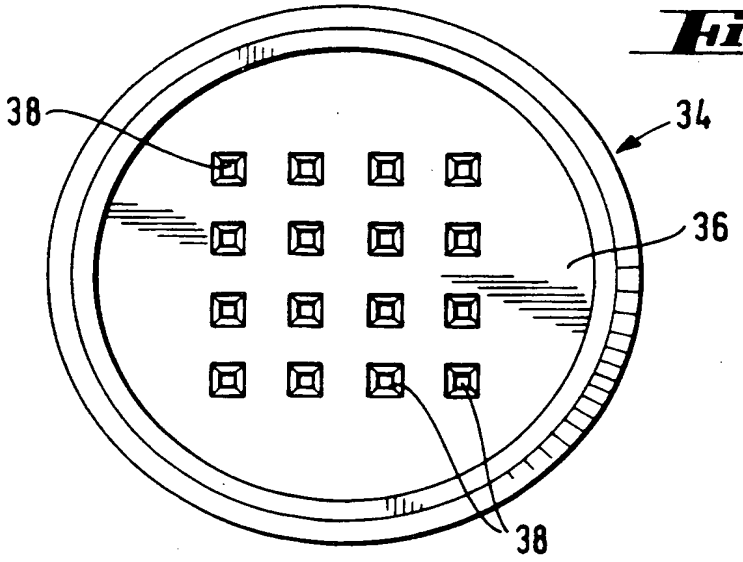


Fig. 6

Fig. 7



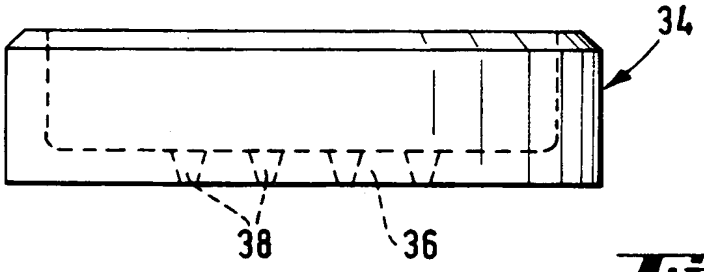


Fig. 8

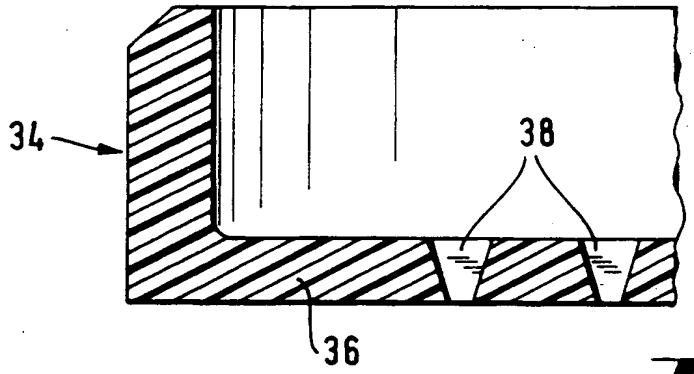


Fig. 9

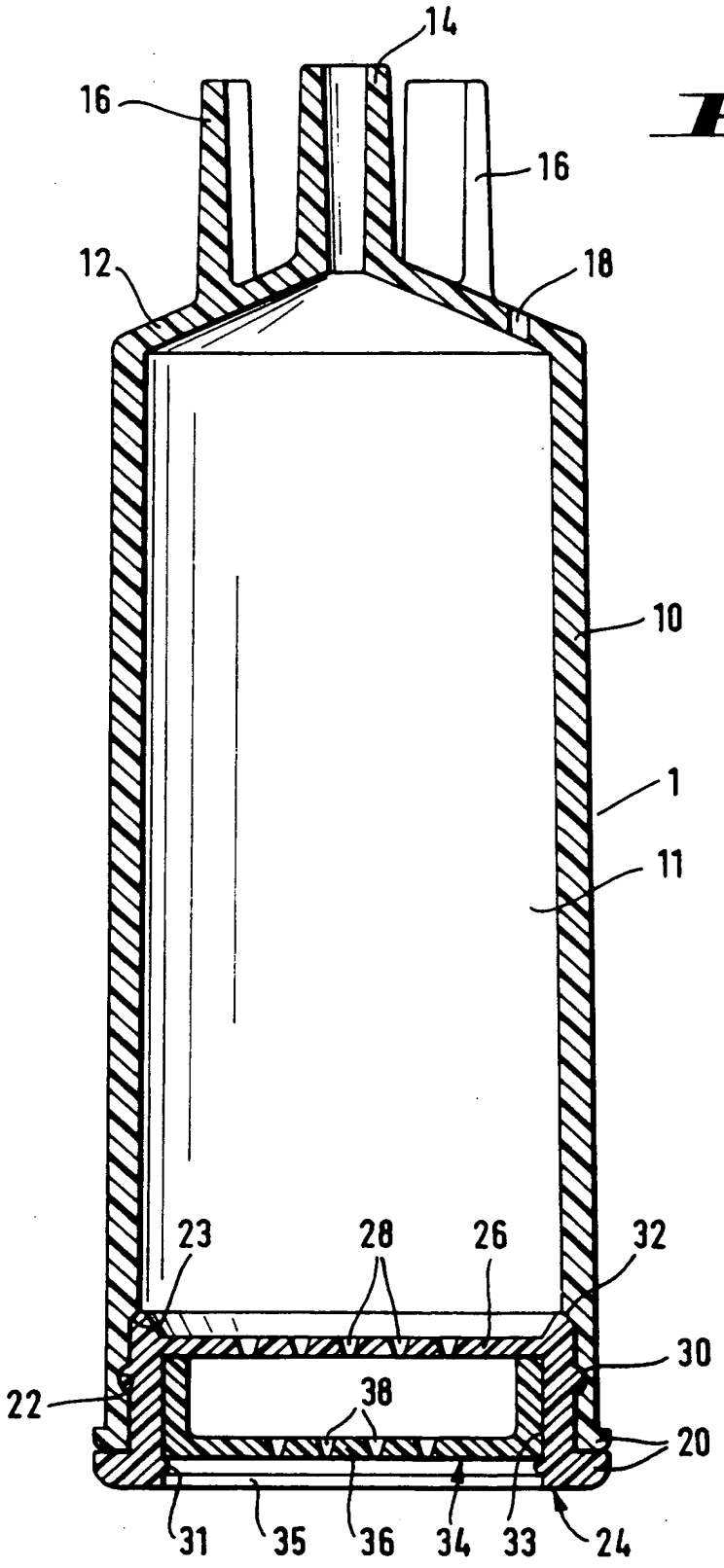
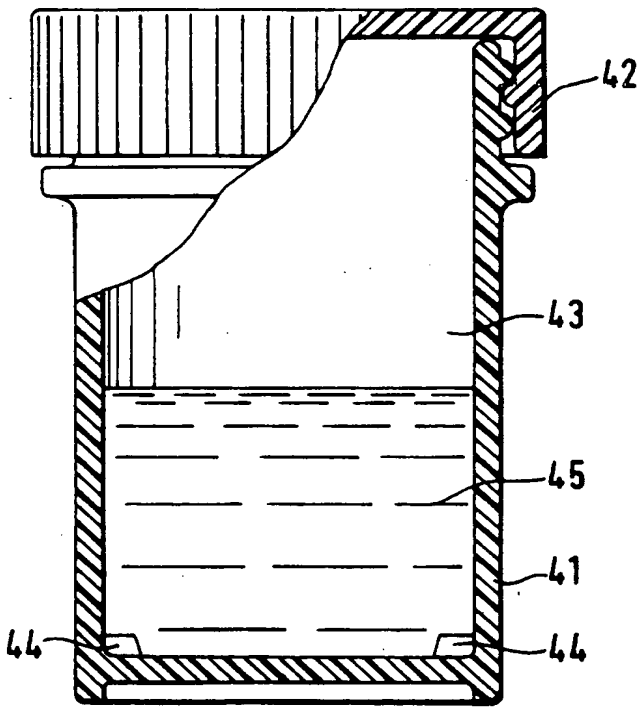


Fig. 10

Fig. 11



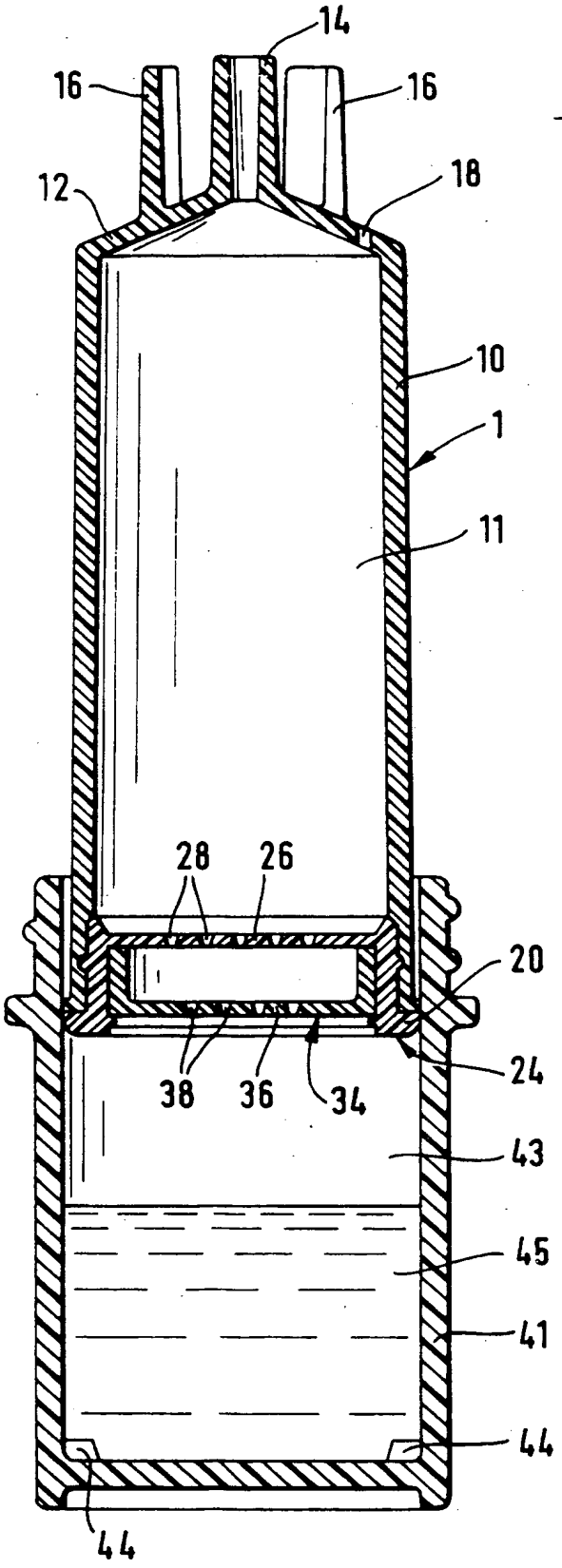
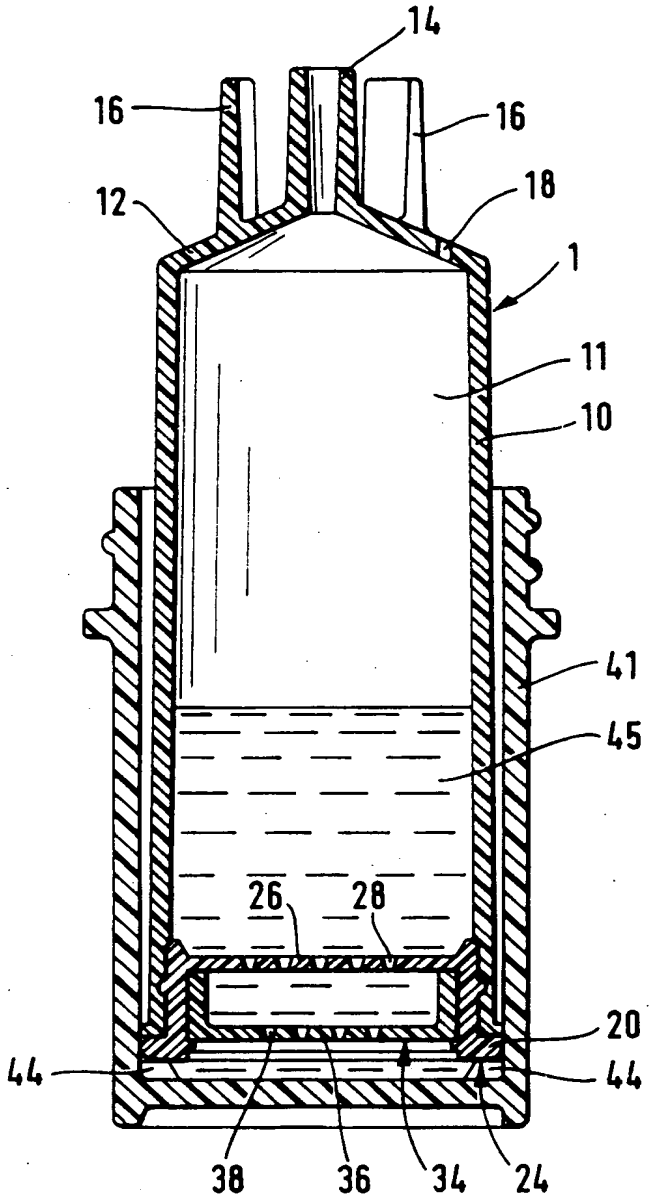


Fig. 12

Fig. 13



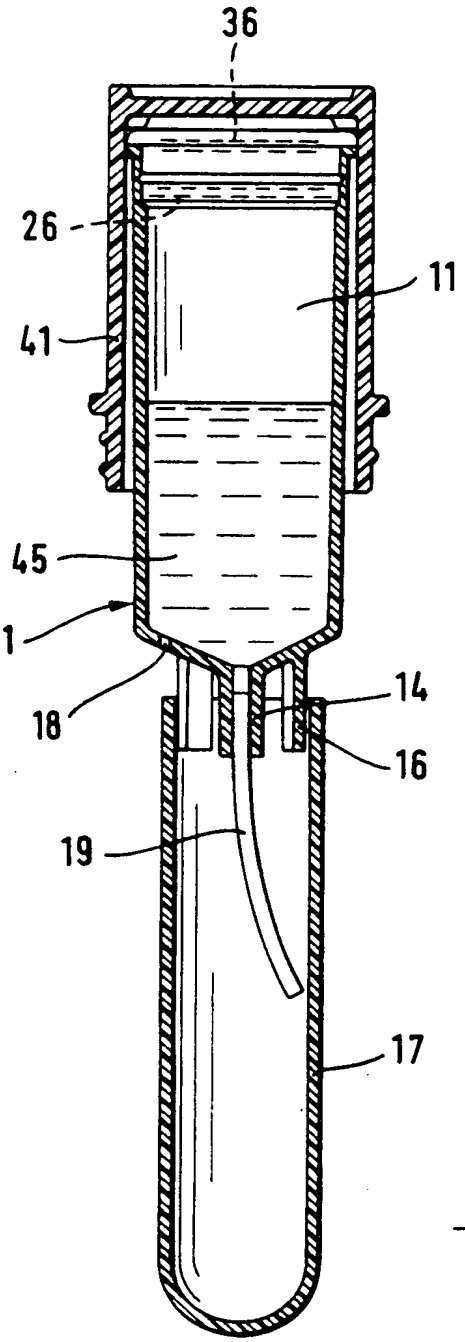


Fig. 14

European Patent Office

EUROPEAN SEARCH REPORT

Application Number
EP 93 11 5303

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.5)
A	US-A-4 350 768 (TIHON) * column 5, line 31 - column 6, line 6 * * claim 6; figure 3 *	1-3	G01N1/28 C12M3/08
A	FR-A-1 270 511 (VELLEY ET AL.) * page 1, left column, last paragraph - right column, paragraph 3 * * page 2, left column, paragraph 6 -last paragraph; figure *	1	
A	US-A-3 941 317 (KANOR) * abstract; claim 1 *	1	

TECHNICAL FIELDS SEARCHED (Int.Cl.5)

G01N
C12M

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
THE HAGUE	3 January 1994	Bindon, C

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
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D : document cited in the application
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